Thursday Poster 083

LC/MS/MS as a Potential Method for Characterizing Bacterial Contamination During Spacecraft Assembly

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Overview

Bacteria cells were processed by a lysis procedure followed by removal of cellular debris and digestion of the cytoplasmic components. LC/MS/MS analyses were performed using a custom built capillary HPLC system coupled to an ion trap mass spectrometer. MS/MS spectra were collected in a data dependent fashion and then matched to known protein sequences using the SEQUEST database search program.

Introduction

Exploration of outer bodies of interest to life's origins requires stringent measures to prevent contamination of these bodies with Earth life forms. Procedures are taken to vigorously clean spacecraft before launch. We are exploring MS to measure contamination during spacecraft assembly. MALDI and ESI-LC/MS create unique protein profiles from bacteria whole cells and cell lysates. Under ideal circumstances, these methods work well, but fail to yield consistent results on samples from different sources and handling techniques. Our more robust approach uses LC/MS/MS of the mixture of peptides obtained from the trypsin digestion of whole cell bacterial lysates

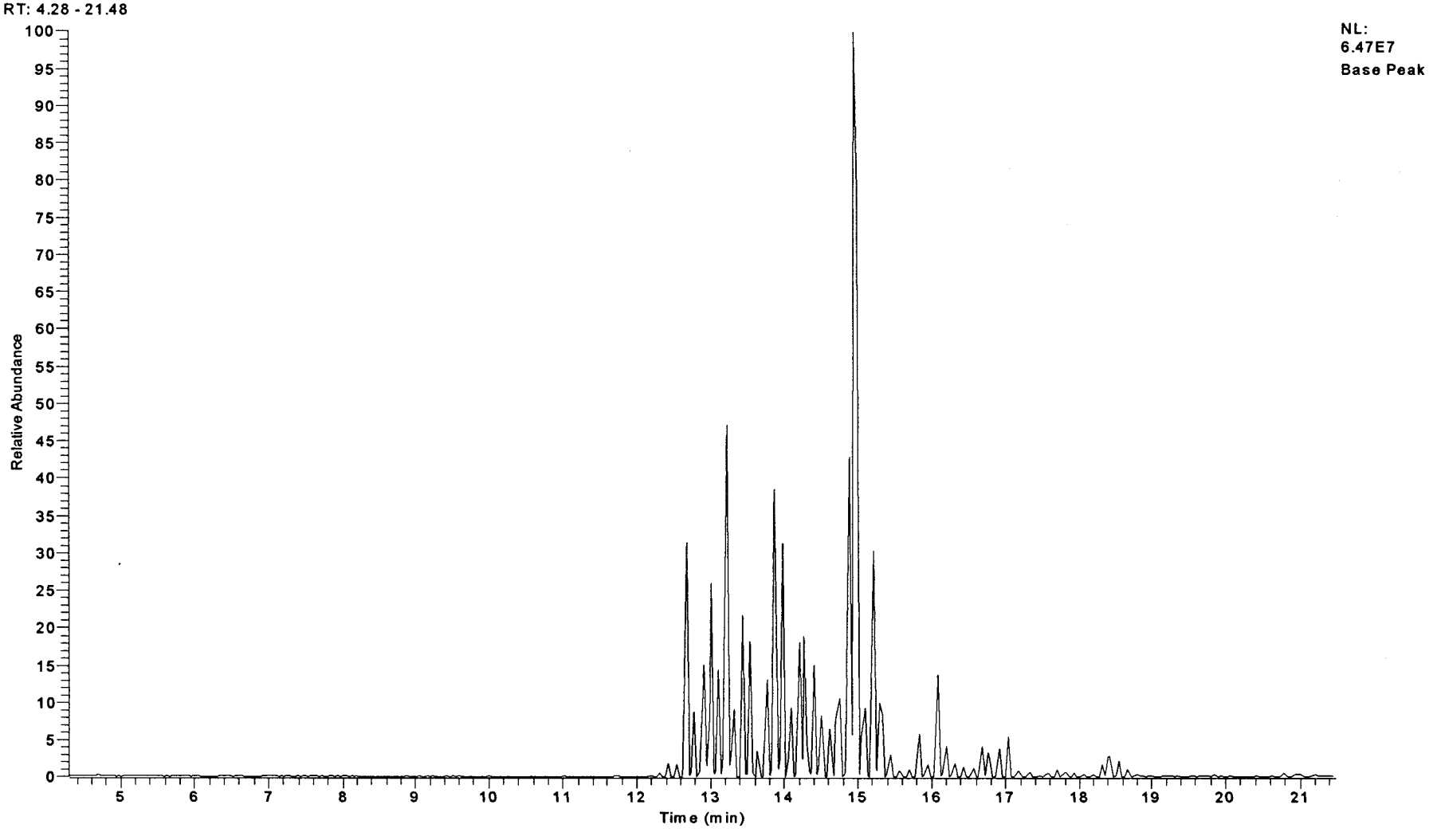
Methods

Freshly grown *B. subtilis* vegetative cell in 0.01% sodium azide were aliquoted into tubes and the solution was adjusted to 20% acetonitrile, 0.1% TFA. Cells were lysed by sonication (20 pulses 5 times at 1 minute intervals) on ice using a Branson sonifier 450. The lysate was centrifuged at 14,000 rpm for 30 minutes in the cold to remove cell debris then lyophilized. The proteins were denatured (urea), reduced (DTT) and alkylated (IAA) and dialyzed using a membrane with a 2K cutoff. An overnight trypsin digestion was performed. LC-MS-MS analysis was performed using a Finnigan LCQ, a custom built gradient loop HPLC and capillary (150u x 5cm) RP C18 column. All ion spectra were screened to eliminate poor MS/MS spectra using winnow program prior to database search.

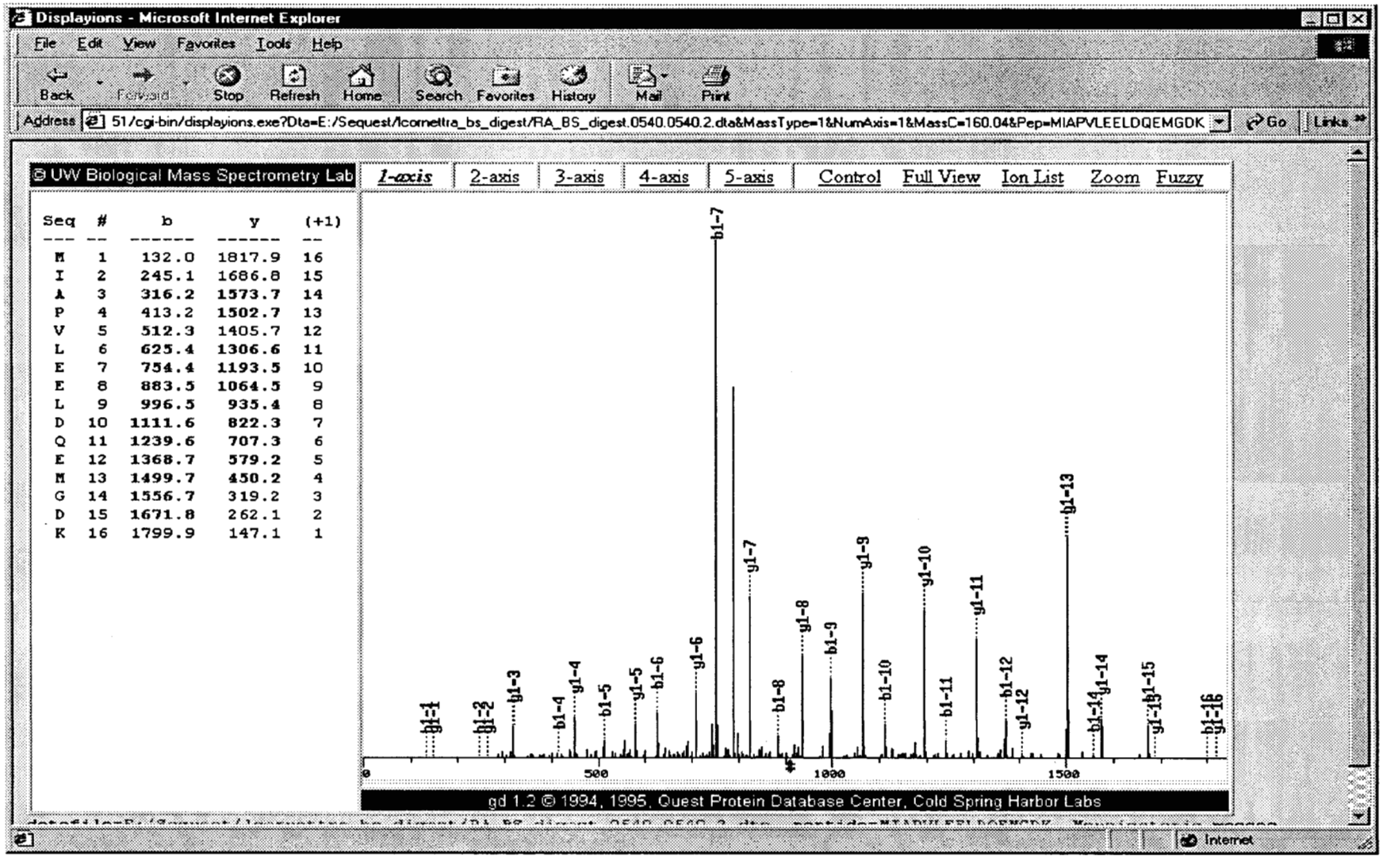
Results

We are in the process of finding optimum conditions for the preparation of bacteria for MS analysis and the data presented are the combined results of 9 runs of varying conditions. Protein profiling did not produce reproducible results using bacteria prepared under different conditions and obtained from different sources. A trypsin digestion however allowed the positive identification of specific *Bacillus subtilis* proteins. Most of the proteins identified are expected to be present in high abundance in the cell. We found that the reduction/alkylation step was required to increase the number of hits by a factor of 2. Peak parking also increased the number of hits by a factor of 2.

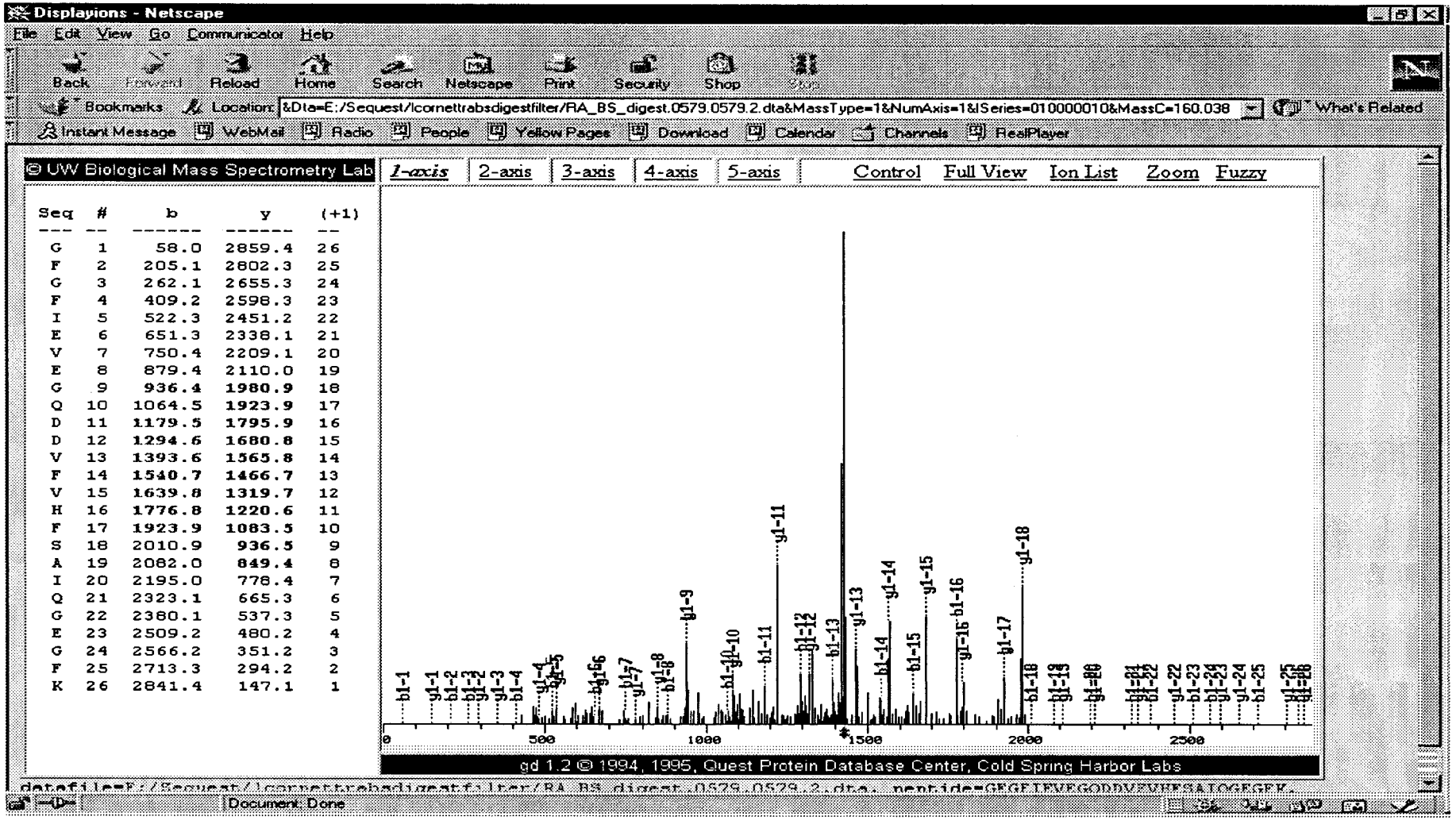
Base Peak of sample. The analysis is fast and most peptides are concentrated in one region.



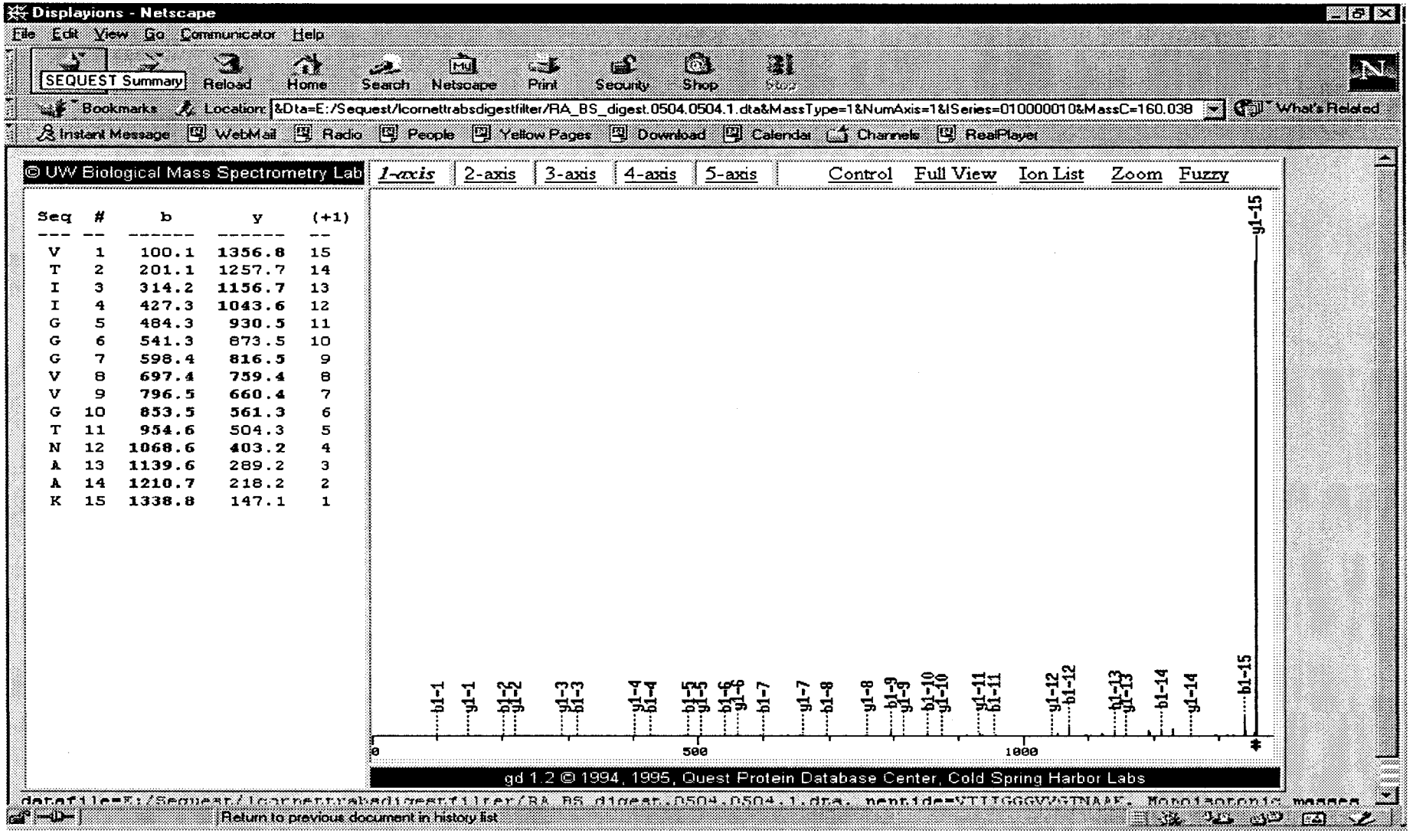
Example of a good MS/MS spectra that matched *B. subtilis* thioredoxin



MS/MS spectra that matched *B. subtilis* cold shock protein CSPB



MS/MS spectra that matched *B. subtilis* alanine dehydrogenase



protein	# times found	organism
owl|f69756|f697	2	BACILLUS SUBTILIS
owl|g69843|g698	4	BACILLUS SUBTILIS
owl|g69985|g699	2	BACILLUS SUBTILIS
owl|h69841|h698	2	BACILLUS SUBTILIS
owl|O08327|GLGD_BACST	2	BACILLUS STEAROTHERMOPHILUS.
owl|p00004|cyc_	7	contaminant
owl|p02394|rl7_	8	BACILLUS SUBTILIS
owl|p02662|cas1	8	contaminant
owl|p02961|sasg	2	BACILLUS
owl|p02968|fla_	5	BACILLUS SUBTILIS
owl|p08877|pthp	9	BACILLUS SUBTILIS
owl|p14949|thio	10	BACILLUS SUBTILIS
owl|p26901|catv	6	BACILLUS SUBTILIS
owl|p27876|tpis	4	BACILLUS SUBTILIS
owl|p28599|ch10	3	BACILLUS SUBTILIS
owl|p32081|cspb	5	BACILLUS SUBTILIS
owl|p38494|rs1h	6	BACILLUS SUBTILIS
owl|p41018|cspb	9	BACILLUS GLOBISPORUS
owl|p49814|mdh_	8	BACILLUS SUBTILIS
owl|p50727|fer_	3	BACILLUS SUBTILIS
owl|p51777|cspd	4	BACILLUS SUBTILIS
owl|p54542|yqje	7	BACILLUS SUBTILIS
owl|p55179|pept	2	BACILLUS SUBTILIS
owl|p80239|ahpc	10	BACILLUS SUBTILIS
owl|p80698|tig_	4	BACILLUS SUBTILIS
owl|p92505|cyc2	3	contaminant
owl|p96707|ydgi	2	BACILLUS SUBTILIS
owl|q08352|dha_	14	BACILLUS SUBTILIS
owl|t01833|t018	2	Caenorhabditis elegans

definition

tellurium resistance protein homolog yceD - Bacillus subtilis
oligoendopeptidase homolog yjbG - Bacillus subtilis
3-hydroxbutyryl-CoA dehydratase homolog ysiB
conserved hypothetical protein yitW
GLYCOGEN BIOSYNTHESIS PROTEIN GLGD
contaminant
50S RIBOSOMAL PROTEIN L7/L12 (BL9) ('A' TYPE) (VEGETATIVE PROTEIN...
contaminant
SMALL, ACID-SOLUBLE SPORE PROTEIN GAMMA-TYPE (SASP)
FLAGELLIN.
PHOSPHOCARRIER PROTEIN HPR (HISTIDINE-CONTAINING PROTEIN).
THIOREDOXIN (TRX).
VEGETATIVE CATALASE
TRIOSEPHOSPHATE ISOMERASE (TIM).
10 KDA CHAPERONIN (PROTEIN CPN10) (PROTEIN GROES).
COLD SHOCK PROTEIN CSPB (MAJOR COLD SHOCK PROTEIN).
30S RIBOSOMAL PROTEIN S1 HOMOLOG.
COLD SHOCK PROTEIN CSPB
MALATE DEHYDROGENASE (VEGETATIVE PROTEIN 69) (VEG69)
FERREDOXIN
COLD SHOCK PROTEIN CSPD
HYPOTHETICAL 39.7 KD PROTEIN IN GLNQ-ANSR INTERGENIC REGION
PEPTIDASE T (AMINOTRIPEPTIDASE) (TRIPEPTIDASE).
ALKYL HYDROPEROXIDE REDUCTASE C22 PROTEIN (GENERAL STRESS PROTEIN 22).
TRIGGER FACTOR (TF) (VEGETATIVE PROTEIN 2) (VEG2).
contaminant
PUTATIVE NAD(P)H NITROREDUCTASE YDGI
ALANINE DEHYDROGENASE (STAGE V SPORULATION PROTEIN N)
wEST02554 Early embryo, Stratagene (cat. #937007) Caenorhabditis elegans cDNA clone CEESW23, mRNA

accession #

contaminant

contaminant

P02968
P08877
P14949
P26901
P27876
P28599
P32081
P38494
P41018
P49814
P50727
P51777
P54542
P55179
P80239
P80698
contaminant
P96707
Q08352

function

unknown; similar to tellurium resistance protein
unknown; similar to oligoendopeptidase
unknown; similar to 3-hydroxbutyryl-CoA dehydratase
unknown; similar to unknown proteins
required for glycogen biosynthesis
contaminant

contaminant

FORM THE FILAMENTS OF BACTERIAL FLAGELLA.
COMPONENT OF THE PHOSPHOENOLPYRUVATE-DEPENDENT SUGAR PHOSPHOTRANSFERASE SYST
REDOX REACTIONS & CATALYZES DITHIOL-DISULFIDE EXCHANGE REACTIONS
SERVES TO PROTECT CELLS FROM THE TOXIC EFFECTS OF HYDROGEN PEROXIDE.
PLAYS AN IMPORTANT ROLE IN SEVERAL METABOLIC PATHWAYS
BINDS TO CPN60 AND SUPPRESSES THE ATPASE ACTIVITY
CAN ACT AS TRANSCRIPTIONAL ACTIVATOR OF COLD SHOCK GENES
RIBOSOMAL PROTEINS
AFFECTS CELL VIABILITY AT LOW TEMPERATURES
[CATALYTIC ACTIVITY] L-MALATE + NAD(+) = OXALOACETATE + NADH
TRANSFER ELECTRONS IN A WIDE VARIETY OF METABOLIC REACTIONS.
[INDUCTION] IN RESPONSE TO LOW TEMPERATURE.
COULD BE A PEPTIDASE
HYDROLYZES A VARIETY OF TRIPEPTIDES CONTAINING N-TERMINAL METHIONINE, LEUCINE, OR PHE
DIRECTLY REDUCES ORGANIC HYDROPEROXIDES IN ITS REDUCED DITHIOL FORM.
INVOLVED IN PROTEIN EXPORT. ACTS AS A CHAPERONE BY MAINTAINING THE NEWLY SYNTHESIZED
contaminant
unknown; similar to NADH dehydrogenase
ASSIMILATION OF L-ALANINE AS AN ENERGY SOURCE THROUGH THE TCA CYCLE DURING

EM (PTS),

NYLALANINE

PROTEIN IN AN OPEN CONFORMATION

Example of a SEQUEST summary file grouped by consensus

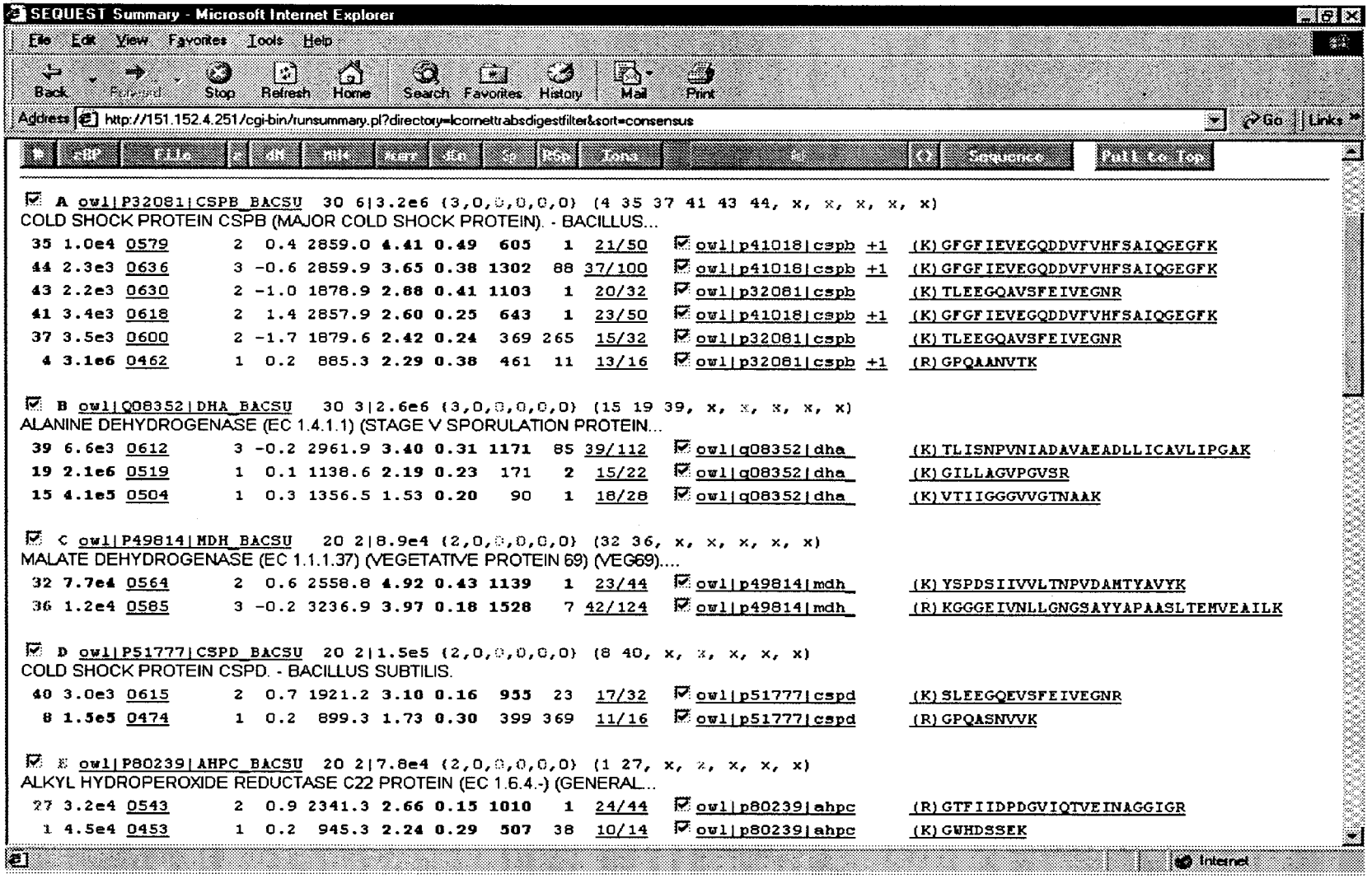
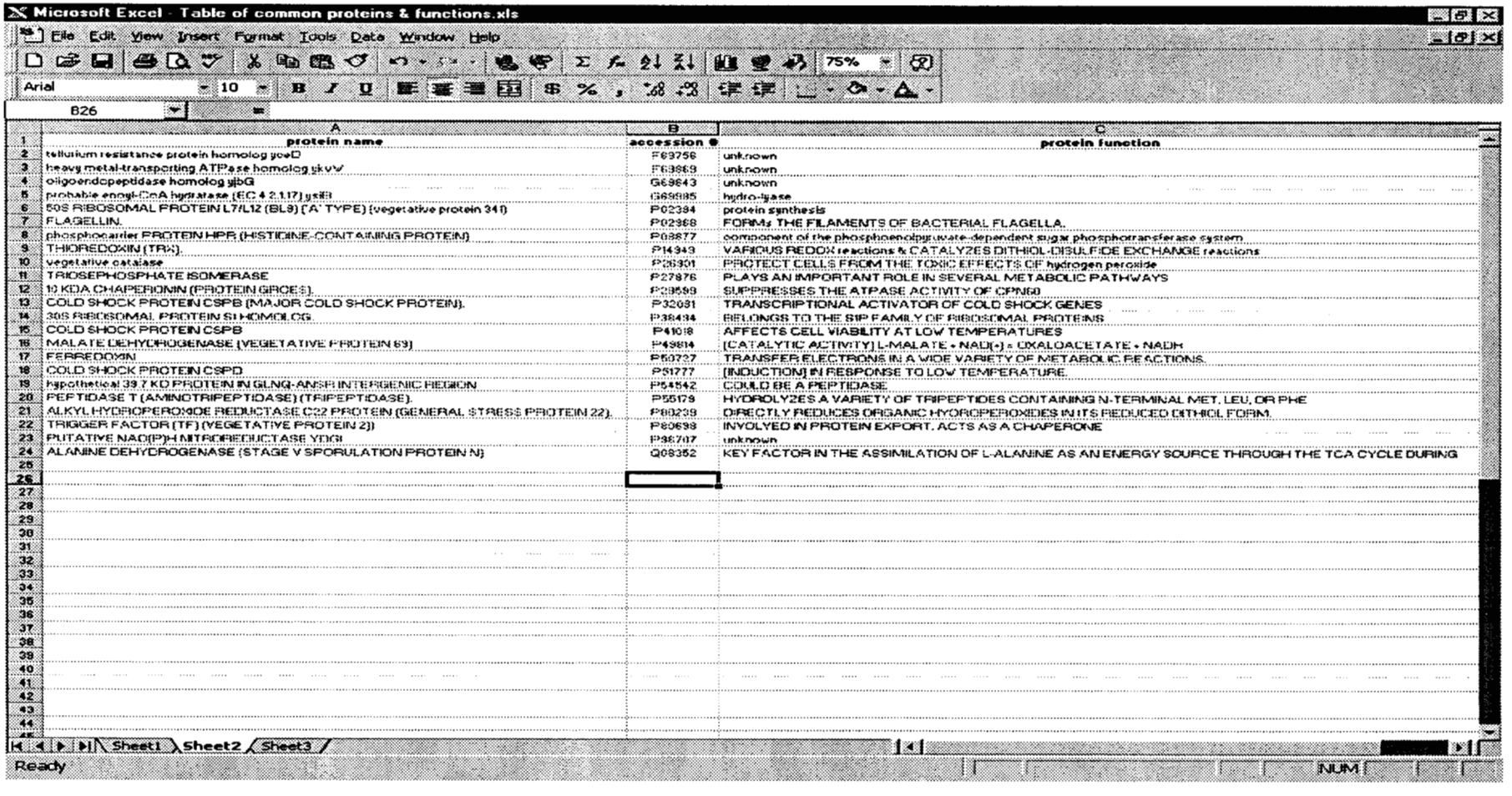


Table of most frequently observed *B. subtilus* proteins and their function



Microsoft Excel - Table of common proteins & functions.xls

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	A	B	C
1	protein name	accession #	protein function
2	tellurium resistance protein homolog yceD	F69756	unknown
3	heavy metal-transporting ATPase homolog ykvW	F69869	unknown
4	oligoendopeptidase homolog yjbG	G69843	unknown
5	probable enoyl-CoA hydratase (EC 4.2.1.17) ysiB	G69985	hydro-lyase
6	50S RIBOSOMAL PROTEIN L7/L12 (BL9) ('A' TYPE) (vegetative protein 341)	P02394	protein synthesis
7	FLAGELLIN.	P02968	FORMs THE FILAMENTS OF BACTERIAL FLAGELLA.
8	phosphocarrier PROTEIN HPR (HISTIDINE-CONTAINING PROTEIN)	P08877	component of the phosphoenolpyruvate-dependent sugar phosphotransferase system.
9	THIOREDOXIN (TRX).	P14949	VARIOUS REDOX reactions & CATALYZES DITHIOL-DISULFIDE EXCHANGE reactions
10	vegetative catalase	P26901	PROTECT CELLS FROM THE TOXIC EFFECTS OF hydrogen peroxide
11	TRIOSEPHOSPHATE ISOMERASE	P27878	PLAYS AN IMPORTANT ROLE IN SEVERAL METABOLIC PATHWAYS
12	10 KDA CHAPERONIN (PROTEIN GROES).	P28599	SUPPRESSES THE ATPASE ACTIVITY OF CPN60
13	COLD SHOCK PROTEIN CSPB (MAJOR COLD SHOCK PROTEIN).	P32081	TRANSCRIPTIONAL ACTIVATOR OF COLD SHOCK GENES
14	30S RIBOSOMAL PROTEIN S1 HOMOLOG	P38494	BELONGS TO THE S1P FAMILY OF RIBOSOMAL PROTEINS
15	COLD SHOCK PROTEIN CSPB	P41018	AFFECTS CELL VIABILITY AT LOW TEMPERATURES
16	MALATE DEHYDROGENASE (VEGETATIVE PROTEIN 69)	P49814	(CATALYTIC ACTIVITY) L-MALATE + NAD(+) = OXALOACETATE + NADH
17	FERREDOXIN.	P50737	TRANSFER ELECTRONS IN A WIDE VARIETY OF METABOLIC REACTIONS.
18	COLD SHOCK PROTEIN CSPD.	P51777	(INDUCTION) IN RESPONSE TO LOW TEMPERATURE.
19	hypothetical 39.7 KD PROTEIN IN GLNQ-ANSP INTERGENIC REGION	P54542	COULD BE A PEPTIDASE.
20	PEPTIDASE T (AMINOTRIPEPTIDASE) (TRIPEPTIDASE).	P55179	HYDROLYZES A VARIETY OF TRIPEPTIDES CONTAINING N-TERMINAL MET, LEU, OR PHE
21	ALKYL HYDROPEROXIDE REDUCTASE C22 PROTEIN (GENERAL STRESS PROTEIN 22).	P80239	DIRECTLY REDUCES ORGANIC HYDROPEROXIDES IN ITS REDUCED DITHIOL FORM.
22	TRIGGER FACTOR (TF) (VEGETATIVE PROTEIN 2))	P80698	INVOLVED IN PROTEIN EXPORT. ACTS AS A CHAPERONE
23	PUTATIVE NAD(P)H NITROREDUCTASE YDGI	P96707	unknown
24	ALANINE DEHYDROGENASE (STAGE V SPORULATION PROTEIN N)	Q08352	KEY FACTOR IN THE ASSIMILATION OF L-ALANINE AS AN ENERGY SOURCE THROUGH THE TCA CYCLE DURING
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Sheet1 Sheet2 Sheet3

Ready NUM

Conclusions

The described procedure depends on obtained fragment ion spectra of sufficient number of the most abundant and readily ionizable peptides present in the cytoplasm for that particular organism. The technique was found to work well for organisms whose genome has been sequenced. For Bacillus subtilis, whose genome is in the database, we obtained specific peptide matches to expected proteins such as chaperonins, metabolic enzymes and ribosomal proteins. While the complex bacterial digestion mixture did not yield the same set of results each time different aliquots or concentrations were analyzed, the majority of high scoring matches were assigned to *Bacillus subtilis* proteins.

We were also able to identify a few hypothetical proteins in the database previously identified by open reading frames. We are unsure at this time whether the presence of these proteins have been detected by any other means. As more bacterial genomes are sequenced, this procedure can be applied to a variety of applications including the analysis of bacteria in distinct microenvironments.

A number of high scoring hits for *C. elegans* was also observed and we later found that this organism is commonly used in the laboratory where the bacteria are being grown hence a possible contaminant for our preparations. The fact that we found this contaminant leads us to believe that our method is sensitive to the presence of other organisms and may be used to identify mixtures of heterogeneous organisms.

References

National Center for Biotechnology Information (NCBI) biomedical databases

Kunst et al (1997) "The complete genome sequence of the Gram-positive bacterium Bacillus subtilus" Nature 390:249

Altschul et al (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

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Krishnamurthy et al (1999) "Liquid chromatography/microspray mass spectrometry for bacterial investigations " *Rapid Commun Mass Spectrom* 1999;13(1):39-49

Moore et al. (2000) "Method for Screening Peptide Fragment Ion Mass Spectra Prior to Database Searching" JASMS 11:422-426

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Curtis Croker for constant modification of our software-controlled HPLC and the peak parking procedure which will be used for any further separations.
John Percy for his programming skills used for the creation of the MoWeD deconvolution software which was initially used in the protein profiling experiments.
Roger Moore for creation of the winnow program which was used as an effective screen for MS/MS spectra.

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